STUDIES IN THE METABOLISM OF APPLES.

III. PRELIMINARY INVESTIGATIONS ON THE EFFECTS OF AN ARTIFICIAL COATING ON THE RESPIRATORY METABOLISM OF GRANNY SMITH APPLES AFTER VARIOUS PERIODS OF COOL STORAGE.

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(Five Text-figures.)

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The preceding paper of this series (Hackney, 1943) dealt with the respiratory metabolism of Granny Smith apples of commercial maturity after various periods of storage at 1°C. It was shown that the concentration of oxygen in the internal atmosphere is probably very important in determining the respiration rate during a certain phase of the history of the fruit. The present paper deals with the metabolism of apples which were in every way similar to those dealt with previously, except that they were coated with a 10% solution of castor oil and shellac in alcohol a few days after removal from store. It was thought that, since the internal oxygen concentration of the fruit is apparently so important, further information about its effects on the respiratory metabolism might be gained by the use of this coating to reduce the internal oxygen concentration artificially. Similar methods of modifying the internal atmosphere have been used before to gain information about the metabolism of fruits. Wardlaw and Leonard (1936) used coatings of vaseline to produce abnormally high concentrations of carbon dioxide in ripening papaws. The first paper of this series (Trout *et al.*, 1942) dealt with preliminary experiments in which the internal oxygen concentrations of Granny Smith apples were artificially lowered by the use of various coatings. The changes brought about by coating may be compared with those which occur naturally, but they take place more quickly and are usually more pronounced.

In addition to their academic interest, it was thought that the investigations undertaken might throw some light on problems connected with the use of artificial coatings to delay ripening in commercial practice.

MATERIALS AND METHODS.

All the experiments were carried out on fruits of commercial maturity, picked from thinned trees at Orange, N.S.W., on 14th April, 1941, and put into cool store at 1°C. on 17th April, 1941. At intervals of 2 to 4 weeks samples of 5 fruits were removed from store to a room kept at a constant temperature of 18·3°C. After one or more observations had been made of respiration rate, composition of internal atmosphere and colour at the new temperature, these fruits were dipped in a 10% solution of castor oil (2 parts) and shellac (1 part) in alcohol. The castor-oil-shellac solution was chosen because it was the most satisfactory of many coatings with which preliminary experiments were made.

The metabolism of uncoated samples removed from store on the same dates has been considered in detail in the preceding paper (Hackney, 1943), but will be further mentioned in this paper when the behaviour of coated fruits is compared with that of normal fruits.

Respiration rate was measured by the Pettenkofer technique and expressed in milligrams per 10 kilograms per hour. The internal concentrations of oxygen and carbon dioxide were determined by the method described in the first paper of the series (Trout *et al.*, 1942). The resistance of the skin to the diffusion of carbon dioxide was calculated by the method used in the first paper of the series. Colouring rate was recorded by comparison with the standards in the "Dictionary of Colour" (Maerz and Paul, 1930).

Table 1 shows the date of removal from store, date of first observation, date of coating and date of final observation for each sample. The first observations were not made on samples 1 and 2 until 6 and 3 days, respectively, after removal from store. In the other samples the first observations were made 1 or 2 days after removal from store.

TABLE 1.

Sample Number.	Date of Removal from Store.	Date of First Observation.	Date of Coating.	Date of Final Observation.	Number of Weeks in Cool Store.
1	6.v.41	12.v.41	13.v.41	27.vi.41	3
2	6.vi.41	9.vi.41	9.vi.41	30.vii.41	7½
3	8.vii.41	9.vii.41	14.vii.41	28.viii.41	12
4	5.viii.41	6.viii.41	11.viii.41	5.ix.41	16
5	2.ix.41	3.ix.41	9.ix.41	23.x.41	20
6	23.ix.41	24.ix.41	30.ix.41	29.x.41	23
7	14.x.41	15.x.41	20.x.41	3.xii.41	26
8	17.xi.41	19.xi.41	25.xi.41	2.xii.41	31

EXPERIMENTAL RESULTS.

Effects of Coating on Respiration Rate.—Curves for mean respiration rates of each sample before and after coating are shown in Figure 1. The corresponding mean respiration rates for the samples which were not coated are also shown. It is clear that samples removed on the same date behaved similarly until one sample was coated. The immediate effects of coating on the respiration rate varied with the time of removal of the fruits from store. In early samples (1 to 4) a decrease in respiration rate occurred. This decrease was evident 24 hours after coating and continued for 6 to 8 days. After the minimum rate had been reached the respiration rate generally remained approximately steady. Meanwhile the respiration rates of the corresponding untreated samples decreased steadily, but slowly, throughout the period of observation. Owing to this decrease, the difference between the respiration rates of the coated and control fruits decreased with time after the first week. When a coated sample first reached its minimum respiration rate, that of the control sample was generally about twice that of the coated sample. One month later, it was only about 1·4 times as great as that of the coated sample.

In the late samples (5 to 8 inclusive) the respiratory behaviour was different from that observed in the earlier samples. In coated samples the respiration rate rose very soon after treatment, the rise being most pronounced in sample 5. The respiration rates of the late control samples also rose after the first few days at 18·3°C., but they did not always rise simultaneously with those of the corresponding coated samples. The peaks reached by control samples were usually higher than those reached by corresponding coated samples. After the initial peak was passed the respiration rates of both coated and control samples fell throughout the periods of observation, and the difference between the respiration rates of corresponding coated and control samples did not decrease with time.

The Effect of Coating on the Internal Atmosphere.—The trends of the mean internal oxygen concentrations of the various coated and control samples are shown in Figure 2. Those of the corresponding carbon dioxide concentrations are shown in Figure 3.

In all samples the internal oxygen concentrations decreased very rapidly after coating, the minimum values usually being observed 24 hours after treatment. The decrease was sometimes followed by a slight recovery.

The minimum internal oxygen concentration varied in different samples. The values did not appear to show any definite trend, and were not related to the internal oxygen concentrations at the time of coating. For instance, samples 1 and 7 both attained a minimum internal oxygen concentration of less than 1% in spite of the fact that the internal oxygen concentration of sample 1 was twice as high as that of sample 7 at the time of coating.

Fig. 1.—Mean respiration rates for 2 sets of 8 samples of Granny Smith apples of the 1941 season. The order of removal from store is indicated by serial numbers. Values for normal fruits are joined by entire lines, those for coated fruits are joined by dotted lines. The observations immediately preceding coating are indicated by arrows.

The effect of castor oil and shellac coatings on the internal carbon dioxide concentration was quite marked. Soon after coating, the concentration rose, the maximum value usually being observed 24 hours after treatment. The peak was very much higher in samples 5 to 8 than in previous samples. After the peak value was attained the internal carbon dioxide concentration fell, usually regaining the pre-treatment level within a fortnight after treatment. The transience of the effect explains its apparent absence reported in a previous paper (Trout *et al.*, 1941).*

* It should also be remembered that some wax coatings have no great effect on the internal carbon dioxide concentrations (see discussion).

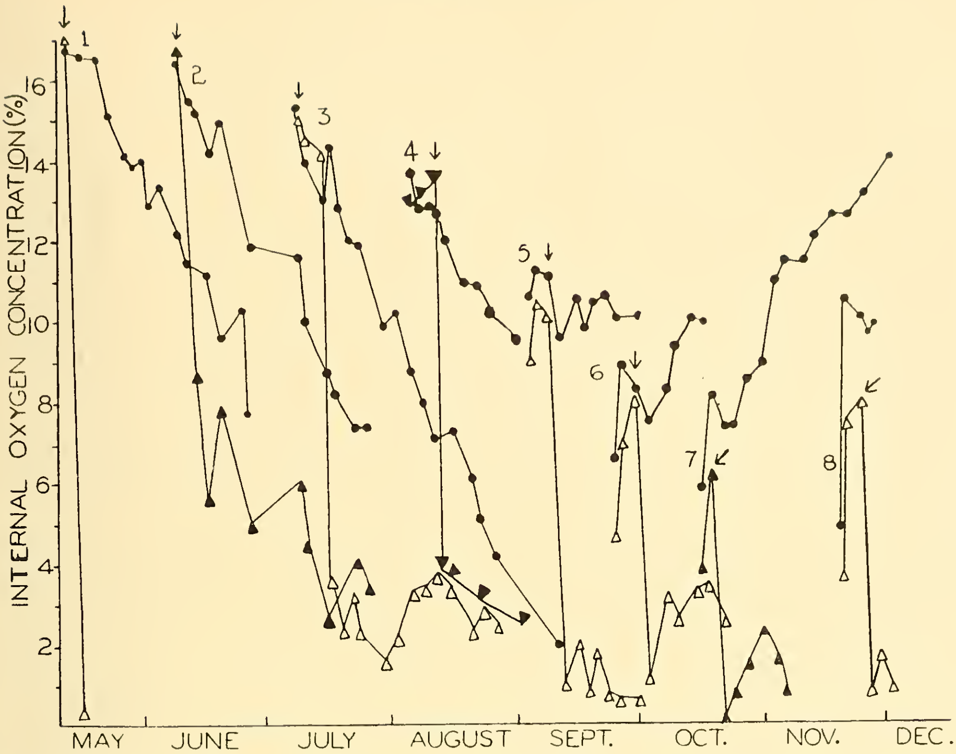

Fig. 2.—Mean internal concentrations of oxygen for 2 sets of 8 samples of Granny Smith apples of the 1941 season. The order of removal from store is indicated by the serial numbers. Values for normal fruits are denoted by black circles; those for coated fruits are denoted by black or white triangles, two symbols being used in the latter case for clearness. The observations immediately preceding coating are indicated by arrows.

Effect of Coating on the Resistance to Gaseous Diffusion.—The resistance of the skin to the diffusion of oxygen was not calculated for all the samples. The calculation of this resistance is based on the assumption that the respiratory quotient does not change. This assumption is open to doubt when applied to coated fruits (see general discussion). In sample 3, which was typical of the early samples, the mean resistance to the diffusion of oxygen apparently increased by more than 200% (3·3 to 10·8 units) after coating. If the respiratory quotient had increased after coating, this apparent increase in r_o would have been an under-estimation of the true value (see method of calculation of r_o in first paper of this series).

Calculations of the resistance of the skin to the diffusion of carbon dioxide (r_c) are independent of the value of the respiratory quotient. For both coated and control samples the trends of the mean resistance of the skin to the diffusion of carbon dioxide are shown in Figure 4. Coating with castor oil and shellac in alcohol brought about a marked increase in r_c. In all samples this increase was at least 200% of the original value, and in some of the samples it was as great as 400%. The high value was not maintained permanently, the r_c falling swiftly during the first two weeks after coating. The final value was still higher than that of the corresponding control fruits. As the r_c of uncoated fruits did not generally decrease, the decrease in r_c of coated fruits must have been due to a change in the coating.

Effects of Coating on the Colouring Rate.—Figure 5 shows the mean colour values of two pairs of samples at various times after removal from store. In all samples colouring rate was greatly retarded by coating. This was in accordance with data previously presented (Trout *et al.*, 1942), where the rate of colouring was probably limited by oxygen supply.

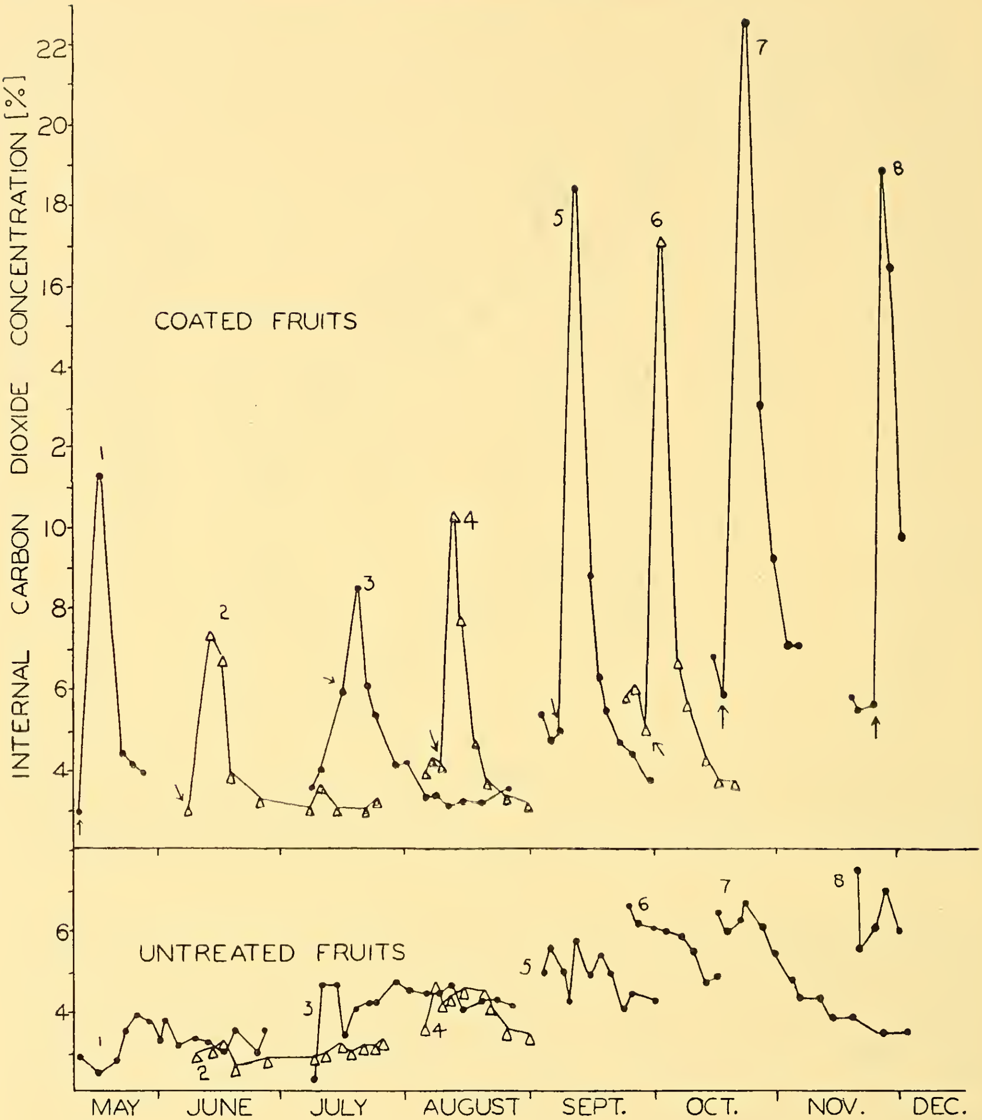

Fig. 3.—Mean internal concentrations of carbon dioxide for 2 sets of 8 samples of Granny Smith apples of the 1941 season. The order of removal from store is indicated by serial numbers. To avoid confusion, different symbols are used for observations on different samples when the curves overlap. The observations immediately preceding coating are indicated by arrows.

Discussion of Results.

The Effects of Coating.—When a coating was applied to a fruit the most immediate effect was probably the increase in the resistance of the skin to gaseous diffusion. This would have brought about two of the observed effects—the decrease in internal oxygen concentration and the increase in the internal carbon dioxide concentration. Either of these might have been responsible for the decrease in the respiration rate. However, the transience of the increase in carbon dioxide concentration makes it improbable that this was the cause of the permanent decrease in respiration rate. It has frequently been observed that Granny Smith apples kept in an atmosphere containing a high concentration of carbon dioxide regained their normal respiration rate when this atmosphere was

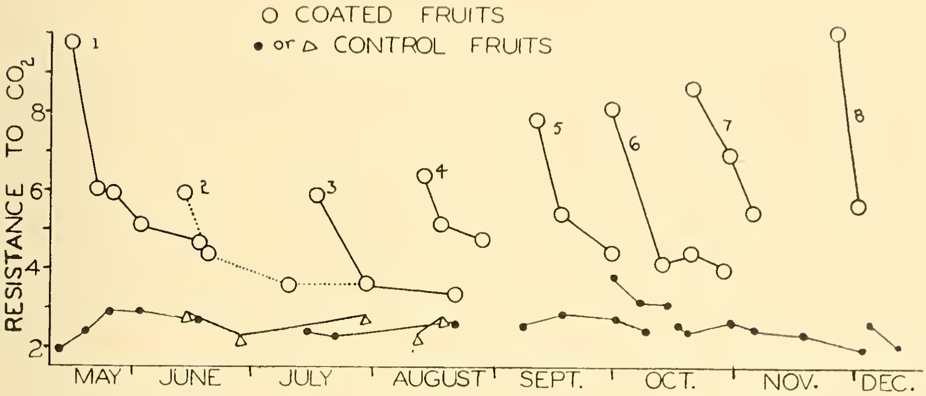

Fig. 4.—Mean resistances of the skin of 2 sets of 8 samples of Granny Smith apples of the 1941 season to the passage of carbon dioxide. The order of removal from store is indicated by serial numbers. To avoid confusion, different symbols are used for different control samples where the curves overlap.

replaced by air. If the increase in internal carbon dioxide concentration had been the cause of the decrease in respiration rate in coated apples, the respiration rate should have recovered its original value when the internal carbon dioxide concentration fell. It seems then that the decrease in respiration rate after coating is more likely to have been due to the reduction of internal oxygen supply.

The Slowness of the Respiratory Decline.—One very puzzling feature was the slowness of the decline in respiration rate after coating. The internal oxygen concentration had reached its minimum value 24 hours after treatment, but periods of 8 or more days elapsed before the corresponding respiration rate attained its minimum value.

When Granny Smith apples were coated with a wax emulsion, it was found that the effects differed in some ways from those of treatment with castor oil and shellac. The wax coating apparently increased the resistance of the skin to the diffusion of oxygen without having any marked effect on the resistance to the diffusion of carbon dioxide. There was a steep fall in internal oxygen concentration, accompanied by an immediate fall to the minimum respiration rate and a slight decrease in the internal carbon dioxide concentration. The wax coating included a continuous phase containing water, which would have facilitated the diffusion of carbon dioxide (Platenius, 1939).

It is possible that in early samples of Granny Smith apples coated with castor oil and shellac the apparent slowness of the respiratory decline might have been due to the gradual release of carbon dioxide trapped inside the fruit at the time of coating, before the respiration rate fell. Blackman (1928) and Wardlaw and Leonard (1936) have already pointed out the importance of "transition effects" brought about when the atmosphere around a fruit is suddenly altered. Wardlaw and Leonard have shown that "under certain experimental conditions the (observed) respiration rate may bear no direct relation to the rate at which cellular respiration is proceeding at that time".

The magnitude of the transition effect on transferring a fruit from air to nitrogen is numerically great (Wardlaw and Leonard, 1936), but its duration is a matter of hours, not days. Had the resistance of the skin of the apples in these experiments remained constant during the first week after coating, the respiratory decline in coated apples would have been too slow to have been explained by gradual escape of carbon dioxide trapped at the time of coating, but it has already been shown that the extra resistance to carbon dioxide imposed by the coating decreased rapidly during the first 10 days after coating. If the resistance to carbon dioxide had been the factor determining the internal concentration and the apparent respiration rate, it would be expected that both these variables would have ceased to decrease when the resistance became approximately constant. The observations set out in Figures 1, 3 and 4 show that this was usually the case.

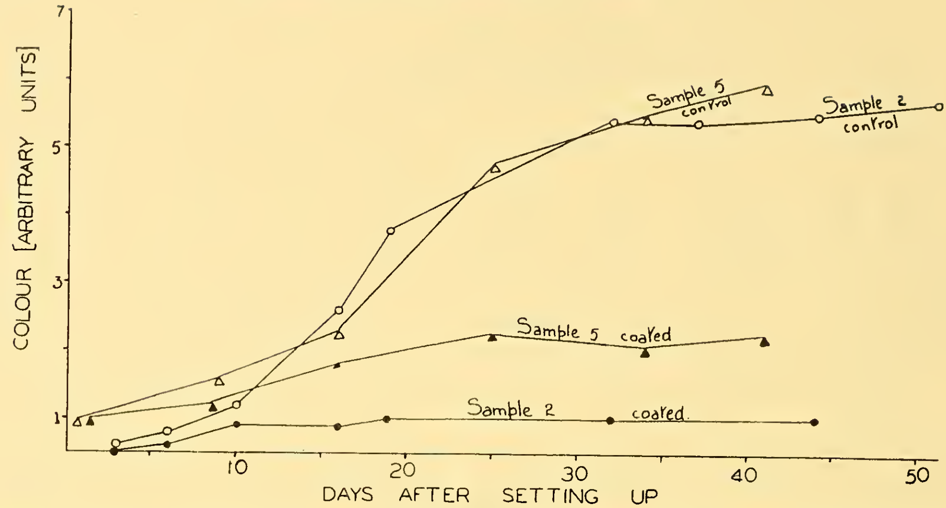

Fig. 5.—Curves showing the mean colour values for 2 samples of untreated Granny Smith apples of the 1941 season and for the corresponding coated samples, at various times after removal from store. Colours 1 to 6 inclusive are given on page 19 of the "Dictionary of Colour" (Maerz and Paul, 1930), where they are denoted by L6, L5, L4, L3, L2 and L1 respectively.

The theory that the apparent slowness of the respiratory decline was due to gradual escape of carbon dioxide trapped inside the fruit on coating was tested numerically, using some of the experimental data. One of the fruits of sample 4 had an internal carbon dioxide concentration of 15·7% twenty-four hours after coating. Five days later the internal carbon dioxide was only 5·3%—a decrease of 10·4% having taken place. The amount of carbon dioxide which would have been given off if the respiration had proceeded at the minimum rate during these 5 days was subtracted from the observed amount given off. The result was approximately 70 milligrams. Had this amount of carbon dioxide been trapped inside the fruit at the time of coating, its escape should have resulted in a decrease of approximately 17% in the internal carbon dioxide concentration (the volume of the fruit being about 150 c.c. and the solubility of carbon dioxide in the cell liquid being taken as 1 c.c./c.c.). The observed decrease was 10·4%. One of the fruits of sample 3 showed a decrease of 3·9% in internal carbon dioxide concentration during the first two days after the maximum concentration had been observed. The theoretical decrease (calculated as above) was 7·0%.

Thus, in each of these fruits more than 50% of the extra carbon dioxide given off (assuming that the respiration rate was actually at its minimum 24 hours after coating) might have been trapped, gas escaping gradually as the resistance of the skin coating decreased. Owing to the possibility of the high concentrations of carbon dioxide having had a depressant effect, the true respiration rates soon after coating might have been even lower than the minimum rates observed later.

The Possibility of Anaerobic Respiration.—A second theory to explain the slowness of the respiratory decline involves the possibility of anaerobic respiration and the formation of an inhibitor. It has been suggested in the preceding paper (Hackney, 1943) that a very small amount of anaerobic respiration may take place in uncoated apples in which the internal oxygen concentrations are low. If the internal oxygen concentration were further decreased by coating, it is not unlikely that the percentage of anaerobic respiration might increase. The decline subsequent to the first 48 hours after coating might have been due to the formation of an inhibitor, such as alcohol. Kidd and West (1933) have shown that for Bramley's Seedling apples the rate of production of carbon dioxide in air is decreased when moderate quantities (0·4% or 0·9%) of ethyl alcohol are introduced into the tissues. It is difficult to explain why the respiration rate ultimately reached a steady value in the first four samples if an inhibitor were constantly being formed. It is possible that after the inhibitor had reached a certain concentration, the rate of its destruction became equal to that of its formation. In the later samples no steady respiration rate was observed.

Alcohol analyses were carried out by Dr. F. E. Huelin, Council for Scientific and Industrial Research, Homebush, using Granny Smith apples comparable with those of sample 6. The technique was that of Fidler (1931). Two days after coating, the fruits contained 0·129% of alcohol; nine days after coating, unpeeled fruits contained 0·301%, while peeled fruits contained 0·264%. The corresponding control fruits contained 0·005% on removal from store, and this value did not increase at 18·3°C.

Alcohol analyses carried out by the writer on fruits of the 1942 season indicated that when samples were taken early in the year there was only slightly more alcohol in treated fruits than in control fruits, but when samples were taken late in the year there was sometimes considerably more alcohol in treated fruits than in control fruits (e.g., 0·068% compared with 0·013%).

It must be remembered that alcohol analyses are not necessarily a true index of anaerobic respiration and that only determinations of the respiratory quotient can be regarded as a true index of the nature of respiration. S. M. Sykes, Department of Botany, University of Sydney, working with coated Granny Smith apples, late in 1942, observed values for the respiratory quotient which were slightly greater than 1·0. On the other hand, control fruits with very low internal oxygen concentrations (below 3%) may have respiratory quotients approximately equal to 1·0. It is unfortunate that no measurements could be made of the respiratory quotients during 1941 and that no alcohol analyses were carried out on the early samples. The results available suggest that anaerobic respiration may play an important part in the metabolism of coated apples, particularly when the fruits are removed from store late in the year.

It is possible that, of the theories put forward to explain the apparent slowness of the respiratory decline, the first may hold for samples removed from store early in the year and the second for those removed late in the year. On the other hand, a combination of the two theories might constitute the true explanation.

Comparison of the Behaviour of Coated and Control Fruits.—In early samples the restriction of the internal oxygen supply consequent upon coating was probably the cause of the decrease in respiration rate. In similar samples of uncoated fruits the resistance to the diffusion of oxygen increased slowly with time, and the respiration rates decreased as the internal oxygen concentration decreased. The effects of coating on the respiration rate were apparently similar to the effects of the natural changes in resistance, but were quicker and more pronounced.

In the later samples the respiratory decline after coating was sometimes preceded by a rise to a peak value. Where this occurred, similar peaks were observed in the respiration curves for the corresponding control samples. This suggests that the peaks in both coated and control fruits might have been due to a common cause (e.g., sudden availability of substrate). On the other hand, the causes of the peak in coated fruits might have been quite different from those of the peak in control fruits. The alcohol concentrations already mentioned indicate a probable difference in metabolism between late-removed coated and control samples as early as two days after coating.

Practical Aspects of the Work.—In the commercial use of coatings to retard ripening, there are two dangers to be considered. The first is that the high concentrations of carbon dioxide observed soon after coating may have some permanent injurious effects on the fruit. The second is that the great reduction brought about in the internal oxygen concentration may cause anaerobic respiration, resulting in the breakdown of the tissues or in alcoholic flavours. Workers of the Council for Scientific and Industrial Research, Homebush, have found that immature Granny Smith apples, coated with castor oil and shellac when the respiration rates were very high, developed high percentages of alcohol.

The changes in the efficiency of the castor oil and shellac coating (as measured by its effect on the resistance to the diffusion of carbon dioxide), which occurs during the first ten days after application, is also an important factor to be considered. The suitability of a coating can not be judged immediately after application. One coating may alter the internal atmosphere to a dangerous extent 24 hours after application, but subsequently allow sufficient recovery to avoid injury. On the other hand, another coating may alter the composition of the internal atmosphere within the limits of safety 24 hours after application, but subsequently become relatively ineffective in retarding ripening.

Summary.

Granny Smith apples of commercial maturity were coated with a 10% solution of castor oil and shellac in alcohol, after various periods of storage at 1°C. The fruits were kept at 18·3°C. and observations were made of respiration rate, concentrations of oxygen and carbon dioxide in the internal atmospheres, and colouring rates. The resistance of the fruit to the passage of carbon dioxide was calculated from the respiration rate and the internal concentration of carbon dioxide. The behaviour of each coated sample was compared with that of a control sample removed from store on the same date.

The effects of coating were depression of the internal oxygen concentration, temporary increase in the internal carbon dioxide concentration, depression of the respiration rate (not always immediately apparent) and retardation of colouring rate. The resistance of the skin to the diffusion of carbon dioxide was increased by coating.

The interrelation of these effects is discussed in detail. In fruits removed from store early in the year the decrease in respiration rate after coating was probably due to the restriction of the oxygen supply. This decrease was comparable with, but more pronounced than, the natural changes which occurred in the resistances of untreated fruits.

In fruits removed from store late in the year the decrease in internal oxygen concentration consequent upon coating may have resulted in the initiation of anaerobic respiration.

Some practical aspects of the work are mentioned.

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